

α 1,2-MANNOSIDASE AND THERAPEUTICAL USES THEREOF

BACKGROUND OF THE INVENTION

(a) Field of the Invention

5 The invention relates to a novel α 1,2-mannosidase and its
therapeutical uses thereof in the treatment of genetic diseases.

(b) Description of Prior Art

α 1,2-Mannosidases are essential for hybrid and complex N-
glycan biosynthesis in mammalian cells (Herscovics, 1999). Following the
10 removal of the glucose residues from the $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ precursor
structure attached to nascent glycoproteins, ER and Golgi α 1,2-
mannosidases catalyse the trimming of the four α 1,2-linked mannose
residues. The subsequent action of GlcNAc transferase I initiates complex
chain formation and yields the substrate for Golgi α -mannosidase II which
15 trims the terminal α 1,3- and α 1,6-mannose residues. In some tissues a
distinct α -mannosidase trims $\text{Man}_5\text{GlcNAc}_2$ to $\text{Man}_3\text{GlcNAc}_2$ prior to the
action of GlcNAc transferase I. Thereafter the N-glycan structure is further
elaborated by Golgi glycosyltransferases.

α -Mannosidases have been classified into two groups based on
20 amino acid sequence homology and on biochemical properties. Class I α -
mannosidases specifically hydrolyze α 1,2-linked mannose residues, and
do not cleave substrates such as p-nitrophenyl- α -D-mannopyranoside.
They require calcium for activity and are inhibited by 1-
deoxymannojirimycin and kifunensine, but not by swainsonine. In contrast,
25 Class II α -mannosidases can cleave α 1,2-, α 1,3- and α 1,6-linked
mannose residues as well as p-nitrophenyl- α -D-mannopyranoside and are
inhibited by swainsonine, but not by 1-deoxymannojirimycin.

 Although several mammalian α 1,2-mannosidases that can
remove up to four α 1,2-mannose residues have been purified and cloned,
30 there is significant biochemical evidence for the existence of highly

specific mammalian enzymes that trim $\text{Man}_9\text{GlcNAc}_2$ to $\text{Man}_8\text{GlcNAc}_2$ isomer B, the form lacking the middle-arm terminal $\alpha 1,2$ -mannose, but mammalian enzymes with this specificity have not yet been purified or cloned. A mammalian ER $\alpha 1,2$ -mannosidase that forms $\text{Man}_8\text{GlcNAc}_2$ isomer B and is not sensitive to 1-deoxymannojirimycin was described in intact UT-1 cells and in rat hepatocytes whereas distinct 1-deoxymannojirimycin-sensitive $\alpha 1,2$ -mannosidase activity that processes $\text{Man}_9\text{GlcNAc}_2$ to $\text{Man}_8\text{GlcNAc}_2$ isomer B was observed in the ER of intact COS cells and in ER and in Golgi rat liver membrane preparations. Up to now the yeast ER processing $\alpha 1,2$ -mannosidase is the only enzyme purified (Jelinek-Kelly and Herscovics, 1988) and cloned (Camirand *et al.*, 1991) that specifically trims $\text{Man}_9\text{GlcNAc}_2$ to $\text{Man}_8\text{GlcNAc}_2$ isomer B.

It would be highly desirable to be provided with the isolation, expression, and properties of a novel human cDNA encoding a Class I $\alpha 1,2$ -mannosidase that specifically converts $\text{Man}_9\text{GlcNAc}$ to $\text{Man}_8\text{GlcNAc}$ isomer B.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide the isolation, expression, and properties of a novel human cDNA encoding a Class I $\alpha 1,2$ -mannosidase that specifically converts $\text{Man}_9\text{GlcNAc}$ to $\text{Man}_8\text{GlcNAc}$ isomer B.

In accordance with the present invention there is provided a human $\alpha 1,2$ -mannosidase enzyme for specifically converting $\text{Man}_9\text{GlcNAc}$ to $\text{Man}_8\text{GlcNAc}$ isomer B in degradation mechanism of misfolded proteins, wherein the enzyme has the characteristics of an enzyme encoded by a cDNA sequence set forth in Fig. 1.

In accordance with the present invention there is also provided the tools to develop specific agonist or antagonist of this particular $\alpha 1,2$ -mannosidase that would not affect the other mannosidases.

The agonist or antagonist may provide for activating or inhibiting for a transient period of time. For example, such an antagonist may be

inhibiting the enzyme for a transient period of time, meanwhile preventing misfolded glycoproteins from being degraded.

In accordance with the present invention there is also provided the potential for a method for the treatment of genetic diseases causing
5 misfolding of proteins in a patient, which comprises administering an antagonist of α 1,2-mannosidase enzyme for transiently inhibiting the enzyme, thereby prevent misfolded glycoproteins from degradation.

For example, such a genetic disease includes, without limitation, cystic fibrosis, emphysema, among others.

10 The misfolded protein for cystic fibrosis is, for example, cystic fibrosis transmembrane conductance regulator (CFTR) (Ward et al., 1995).

The misfolded protein for emphysema is, for example, alpha1 antitrypsin.

15 For the purpose of the present invention the following abbreviations are defined below.

ER endoplasmic reticulum;

RT reverse transcriptase;

ORF open reading frame;

20 RACE rapid amplification of cDNA ends;

GSP gene specific primer;

YPD yeast peptone dextrose;

BMGY buffered glycerol-complex;

BMMY buffered methanol complex;

25 HPLC high performance liquid chromatography.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the nucleotide and deduced amino acid sequence of the human α 1,2-mannosidase cDNA;

30 Fig. 2 illustrates Northern blot analysis of human α 1,2-mannosidase expression;

Fig. 3 illustrates the expression of the recombinant α 1,2-mannosidase in *P. pastoris*;